Poliomyelitis and Other Enteric Viruses in Sewage

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The frequent occurrence of poliomyelitis and Coxsackie viruses in sewage from two New York State areas adds another item to the recorded epidemiology of these infections. The findings are dependent on isolation technics used.

While seeking ways to improve the detection of viruses in water and sewage, the sewage from two areas in New York State was examined regularly for enteric viruses in 1954 and 1955. The agents found and the circumstances of their isolation are summarized here.

Materials and Methods

Three hundred and eight sewage samples were tested for pathogenic agents in newborn mice. Two hundred and eight of them (Series B) were tested for cytopathogenic agents in HeLa cells [1] and the remaining 100 (Series A) in monkey kidney epithelium [2] cultures. Series B samples were raw sewage from communities of various socioeconomic levels in Erie County and Series A samples, sewage from stages of treatment in Albany and environs. The samples were collected by suspending cheesecloth swabs [3] in flowing sewage for two days (Series A) or for seven days (Series B). The expressed liquid of the swabs was adsorbed onto and eluted from ion exchange resin,[4] treated with antibiotics, and stored in a dry ice chest at —45° C until tested. Etherization [5] and centrifugation of the thawed eluates before inoculation into tissue cultures were helpful in reducing toxicity.

The eluates were inoculated into newborn mice and into tissue culture tubes. Eight one-day-old mice were inoculated subcutaneously with 0.03 ml of eluate and eight intraperitoneally with 0.05 ml. Mice paralyzed or spastic during the 14-day observation period were killed and their tissues harvested for confirmatory passage. Pathologic examination and serologic identification were carried out on confirmatory passage material.

Three four- to seven-day-old tissue cultures containing 0.5 ml maintenance [1] or 199H [6] solution were inoculated with 0.1 ml amounts of the eluates and were incubated at 32° or 37° C. Before inoculation cultures were rinsed at least twice to remove traces of serum. Fluids from cultures which showed signs of degeneration during the six- to 10-day observation period were subcultured. Serologic typing was carried out with second- or third-passage fluids.

Results

When the data are organized to illustrate the kinds of agents found (Figures 1 and 2) it is clear that most of the isolates were identified as poliomyelitis or Coxsackie viruses. Agents isolated in mice were Coxsackie viruses and those in tissue cultures were poliomyelitis or Coxsackie viruses, or unknown. Poliomyelitis viruses were found in 21 per cent of the samples; Coxsackie

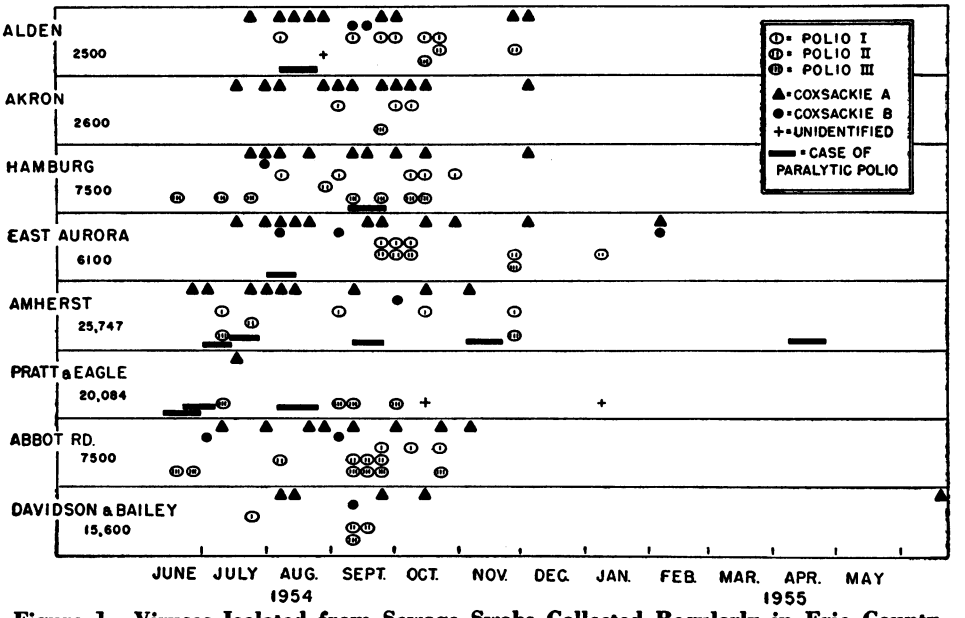

Figure 1—Viruses Isolated from Sewage Swabs Collected Regularly in Erie County, N. Y., from June, 1954, Through June, 1955

viruses in 42 per cent; both poliomyelitis and Coxsackie viruses in 13 per cent; and unidentified agents in 3 per cent.

Both poliomyelitis and Coxsackie viruses occurred in similar seasonal patterns. Thus, neither was found until early summer and appeared regularly through fall. Of the poliomyelitis viruses, Type 1 was encountered most frequently (Table 1). Type 3 was also commonly encountered in Erie County. Type 2 was the least often found. In two communities (Figure 1), Type 3 poliomyelitis virus was apparently the predominant type. Mixtures of two or more poliomyelitis types, of Coxsackie types, or of poliomyelitis and Coxsackie viruses occurred in many of the samples.

Coxsackie A strains were present throughout the season; Coxsackie B strains were less frequent. Different serologic types of Coxsackie B strains were found in the two areas, B_2 and B_4 in samples from the Albany area, and B_3 and B_5 in Erie County samples.

The samples from Erie County were collected in a manner designed to determine relationships between the socioeconomic level of a community, virus presence in sewage, and incidence of paralytic disease. It is difficult from the data given in Figure 1 to recognize relationships among these factors. Alden, a rural community with a small con-

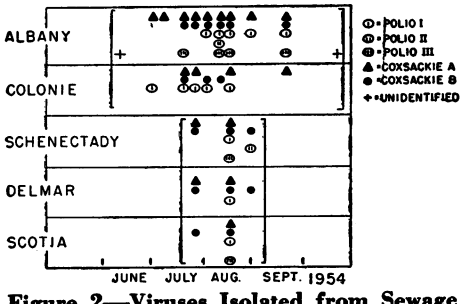

Figure 2—Viruses Isolated from Sewage Swabs Collected Regularly in and Around Albany, N. Y., 1954. Periods of Collection in Brackets

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Table 1—Number of Samples Containing Poliomyelitis Virus

	Type		
	1	2	3
Erie County (Series B)	25	15	23
Albany (Series A)	23	2	8

tributing population, had a poliomyelitis virus frequency in sewage equal to that of East Aurora, a small town with double the population. It was similar also to that of Amherst, a suburban community with five times the number of reported paralytic cases. A neighborhood with frequent poliomyelitis isolations from sewage, Abbott Road, had no reported paralytic cases, and Amherst had a high case rate with relatively few poliomyelitis virus isolations from its sewage.

Several conjectures, however, may be made from the isolations listed in Figure 1. The isolation of poliomyelitis viruses from three communities where no paralytic cases were reported suggests that carriers, silent infections, or subclinical cases contribute to the agents found in sewage. The failure to isolate Coxsackie virus consistently from one community, Pratt and Eagle, cannot be attributed to toxicity since poliomyelitis virus Type 3 was isolated several times and the coliform count of the samples (MPN/100 ml of 3,000,000) was normal. In addition to domestic sewage, this sampling point received brewery wastes.

Table 2—Virus Types Isolated from Primary Sewage Treatment Plants

		Type of Sewage		
Virus		Raw	Effluent	Outfall Points
Poliomyelitis	1	+	+	+
	2	+	+	
	3	+	+	
Coxsackie	A	+	+	+
	B			
	2	+	+	
	3	+		
	4	+		
Unknown		+		

It is well known that certain sewage treatments do not destroy viruses. The isolations listed here were from raw sewage, effluents, and outfall points of primary treatment plants and from the filter effluent and outfall points of secondary treatment plants. Indeed, the enteric viruses isolated from the effluents of primary treatment plants were many and varied (Table 2). Isolations were occasionally made from receiving streams several feet from the outfall. No isolations were made from the chlorinated effluent of a secondary treatment plant. Agents were found, however, in its receiving stream, yards away from the outfall (Table 3). This anomaly was assigned to the drainage from sludge drying beds which entered the stream between two sampling points.

When the isolations are analyzed according to the host employed (Table 4)

Table 3—Isolation of Viruses from Stream Receiving Chlorinated Effluent of Secondary Sewage Treatment Plant

	Virus Isolated				
				Downstream from Outfall	
Collection Date—1954	Raw Sewage	Filter Effluent	Final Effluent	50′	75′
July 19–21	AP_1	B_2	..	....	B_2
August 2–4	B_2		..	$B_2 P_1$	..

Table 4—Virus Strains Isolated from 308 Sewage Specimens Inoculated into Newborn Mice, and Monkey Kidney or HeLa Cell Cultures

Agent	Number of Strains Isolated					
	Series B			Series A		
	Mice Only	Monkey Kidney Tissue Only (100 Specimens)	Simultaneously in Mice and Monkey Kidney	Mice Only	HeLa Cells Only (208 Specimens)	Simultaneously in Mice and HeLa Cells
Coxsackie A	41	0	0	63	0	0
Coxsackie B	9	10	10	6	3	1
Poliomyelitis	0	33	0	0	64	0
Unknown *	0	2	0	0	3	0
All agents	50	45	10	69	70	1

* Not mouse pathogenic; not poliomyelitis 1, 2, or 3; not Coxsackie B1-5.

the effect of isolation method on numbers and kinds of agents found becomes apparent. The isolates were obviously different in mice (Coxsackie A and B viruses) from isolates in tissue cultures (Coxsackie B and poliomyelitis viruses). Conversely, no Group A Coxsackie viruses were isolated in tissue cultures and no poliomyelitis viruses were isolated in mice.

Differences resulting from the use of the two kinds of tissue culture, HeLa cells and monkey kidney tissue, are less obvious. Poliomyelitis viruses were isolated in HeLa cultures from 22 per cent of the samples and in monkey kidney epithelium from 28 per cent, suggesting like sensitivities of the two cultures to poliomyelitis virus. Coxsackie viruses were isolated more frequently in monkey kidney tissue, on the other hand, than in HeLa cells. While these data, however, do not clearly demonstrate a difference in sensitivity of the two tissue culture types—Coxsackie viruses were isolated more frequently also in the mice inoculated with Series A samples—established strains of Coxsackie viruses are known to grow more readily in monkey kidney tissue than in HeLa cell cultures.[7, 8] Consequently, serologic Coxsackie B types isolated from the two areas may suggest a real difference in predominant types or an artificial one arising from the use of tissue cultures of different sensitivities.

That the differential in number of agents isolated in monkey kidney epithelium over that in HeLa cells is not greater than found is the more surprising when note is made of a basic difference in the two culture types, i.e., a relatively wild population versus a stable one.

Coxsackie B viruses isolated in mice were not always isolated simultaneously in tissue culture, and vice versa (Table 4). Although the recognition of Coxsackie viruses is based on signs of disease in newborn mice,[9] several samples yielding agents in tissue culture identified serologically as Coxsackie B strains were not pathogenic for mice. Strain differences may be involved here as observed by Sickles, et al.,[7] or difficulty in recognizing lesions in mice, or in some, overgrowth by more rapidly growing Group A strains. On the other hand, several samples yielding agents in mice identified as Coxsackie Group B viruses were not cytopathogenic. Strain differences may be responsible or, in

some, overgrowth of the slower incubating Coxsackie B strains by poliomyelitis virus.

Discussion

Sewage, of course, is a special source of enteric pathogens, and the samples described, collected chiefly during the summer, are a further selection. Because it is a rich source of viruses, sewage is suitable material for a comparison of methods for their isolation.

All but 2 per cent of the agents isolated were identified as members of Coxsackie Group A (43 per cent), or Coxsackie Group B (16 per cent), or as poliomyelitis virus (40 per cent). Herpes, adenoviruses, and others which at times have appeared in fecal specimens were not recognized. The few unidentified agents may eventually prove to be well known pathogens. Other viruses may have been present but not recognized. Johnsson,[10] for example, estimates the efficiency of mouse tests for Coxsackie B viruses to be only 31–60 per cent.

The frequency of mixtures deserves attention, since mixtures have often confused the identification of Coxsackie viruses. Mixtures might be expected to be more common in sewage than in individual fecal specimens, but they occur in both and this fact must be borne in mind. Several of the poliomyelitis strains at first appeared to be unknown agents; by further serologic testing they were found to be mixtures of at least two strains. Mixtures were resolved by successive neutralization tests with antisera for each or all of the known poliomyelitis virus type. Successive neutralization tests were carried out on fluids from initial tests in which the appearance of cellular lesions was delayed for so short a period as 24 hours.

The failure to isolate Coxsackie Group A viruses in tissue culture in this experience does not prove a complete distinction between mice and tissue cultures as indicators for the isolation of Coxsackie viruses. Group A-9 strains, for example, have frequently been isolated in tissue culture, and certain other Group A strains are known[8] to grow in tissue cultures. Group B strains were isolated as successfully in tissue culture as in mouse tests, 26 being isolated in mouse and 24 in tissue culture tests. The B strains isolated in HeLa cells in the present study, B-3 and B-5, are among the three known from earlier work[7,11] to be most readily propagated in HeLa cells. This, too, is not an absolute difference. Hummeler[12] has isolated a Coxsackie B-2 strain in HeLa cells, and two B-4 strains recently received from Dr. A. J. Rhodes of the Hospital for Sick Children, Toronto, have grown well in HeLa cells.

The five unidentified agents were isolated in tissue cultures. They were not neutralized by poliomyelitis or Coxsackie B antisera, nor were they mouse pathogenic.

These comparisons indicate that the choice of host is a critical factor in the isolation and identification of agents. Tissue culture hosts are excellent for isolation of poliomyelitis viruses. Their identification in tissue culture may be confused, however, by the simultaneous occurrence of other agents, such as some of the Coxsackie viruses. The use of tissue cultures exclusively, furthermore limits the variety of agents which may be found, for example, in sewage. Newborn mice, on the other hand, are of no value for isolations of poliomyelitis virus; for Coxsackie A virus isolations, however, they exceed that of tissue culture hosts. To exhaust the possibilities of the search for agents (if that is an object) isolation in several hosts is required.

The observations suggest also that unknown viruses, if enteric in nature, may sometimes be identified as mixtures of poliomyelitis viruses or of Coxsackie

viruses, or of poliomyelitis and Coxsackie viruses, if the proper combination of hosts is employed and judicious use of antisera made; and that the virologists' searching is like the prophet's vision—only what it foresees it finds.

Summary

Samples of sewage from two areas in New York State were examined for viruses in parallel tests in newborn mice and tissue cultures. Poliomyelitis and Coxsackie viruses were frequently isolated during the summer and fall from both untreated and treated sewage. No correlation was noted between poliomyelitis viruses in sewage, reported paralytic cases, and socioeconomic status of the community.

The number and types of agents isolated differed according to the isolation method used. Coxsackie A viruses were isolated in mice only, poliomyelitis viruses in tissue culture only, and Coxsackie B viruses in both tissue culture and mice. Coxsackie B isolations were made as frequently in mice as in tissue cultures and they were isolated more frequently in monkey kidney tissue than in HeLa cell cultures. Many mixtures of types were encountered and a few agents were unidentified.

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